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(57) Abstract

Constructs comprising a human APP or A4CT DNA sequence encoding mutations which lead to a higher ratio of $\beta A4_{1-42}/\beta A4_{1-40}$ than wild type and their use in the production of transgenic animals developing amyloid plaques as a model of Alzheimer's disease.

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TRANSGENIC ANIMALS WITH MUTANT HUMAN APP OR A4CT SEQUENCES

This invention relates to modified amyloid precursor proteins and their use in the production of transgenic animals.

The main protein component of the amyloid plaques found in the brain of Alzheimer's disease (AD) patients is βA4, a 4 kDa peptide consisting of mainly forty and forty-two residues ($\beta A4_{1-40}$, $\beta A4_{1-42}$) being derived from the amyloid precursor protein (APP). By an enzyme called β-secretase APP can be cleaved at the N-terminus of βA4 generating a soluble APP and the C-terminal fragment A4CT (C99). This 99 residue long membrane protein A4CT (ref. 1) which is the direct precursor for βA4 contains the entire βA4 domain, the membrane domain and the cytoplasmic tail of APP. Alternative processing of APP in a post-Golgi-compartment by a protease termed α-secretase leads to the cleavage of APP within the βA4 domain yielding secretory APP and the transmembrane fragment p3CT which is the direct precursor for p3.

Both C-terminal fragments of APP, A4CT and p3CT, are cleaved within the membrane domain by a γ-cleavage activity, thereby releasing βA4 and p3 into the medium (refs. 2, 3). In cells expressing wild type APP the site of γ-cleavage is mainly the peptide bond Val(40)-Ile(41)of A4CT and to a minor extent the bond Ala(42)-Thr(43). In cells expressing APP with the Familial AD linked mutations at Val 717 (based on APP_{770}, Val 46 of A4CT) an increased γ-cleavage occurs behind Val(42), thus producing larger amounts of $\beta A4_{1-42}$ (ref. 4).

Transgenic mice expressing A4CT have been produced using the human APP promoter (ref. 5), the human thy-1 promoter (ref. 6) and the JC viral early region promoter (ref. 7). Numerous promoters have been used in conjunction with the full length APP cDNA (ref. 12). Generation of transgenic mammals bearing APP derived DNA sequences are also described in WO93/14200 (TSI Corporation), WO91/19810 (California Biotechnology Inc), WO93/02189 (University of California), WO89/00689, WO92/06187 (The Upjohn Company), EP0451700 (Miles Inc.), WO92/13069 (Imperial College of Science Technology and Medicine) and WO89/06689 (McClean Hospital Corporation).

Results obtained depend upon the source of the promoter and the protein coding sequence used. However in all cases described to date the nature of the APP-immunoreactive deposits did not resemble the clinical situation and, with the exception of the model described in reference 13 such transgenic animals have not been found to be faithful model systems for Alzheimer's disease.

The $\beta A4_{1-42}$ peptide is the major subunit of amorphous and neuritic plaques in Alzheimer's disease.

Applicants have found that recombinant cells expressing A4CT carrying certain mutations in the A4CT amino acid sequence lead to a higher ratio of $\beta A4_{1-42}/\beta A4_{1-40}$ than wild type and such mutant proteins and coding DNA are therefore useful in the production of transgenic animals developing amyloid plaques as a model of Alzhemer's disease. It will be understood that references herein to A4CT, $\beta A4_{1-42}$ and $\beta A4_{1-40}$ include all N-terminal variants produced by alternative cleavage during processing.

According to the present invention there is provided a non-human transgenic mammal whose cells contain a construct comprising a human APP or A4CT DNA sequence encoding a mutation selected from:

(i) T43A, T43S, T43G, T43V, T43L, T43I or T43F; and

(ii) Insertion in the transmembrane domain (residues 43 to 52) of a sequence of 1 to 10 amino acid residues;

(numbering relative to A4CT) operably linked to a promoter sequence.

In a preferred aspect the construct encodes mutations (i) and (ii).

Mutation (i) is preferably selected from T43A and T43S.

Insertion (ii) is preferably located between residues 42 and 53, more preferably between 46 and 53. In a preferred embodiment the insertion is located between T48 and L49. The residues for insertion (ii) are preferably selected from F, I, G, Y, L, A, P, W, M, S, T, N and Q. The insertion (ii) is preferably 2 to 6 residues long. In a preferred embodiment the insertion (ii) is LV.

In a further preferred aspect the construct additionally encodes a mutation selected from:

V46F, V46I, V46G, V46Y, V46L, V46A, V46P, V46W, V46M, V46S, V46T, V46N or V46Q. In a preferred embodiment the additional mutation is V46F.

In a preferred embodiment the construct further encodes the APP signal sequence (APP residues 1 to 17) immediately upstream of the APP or A4CT DNA sequence. Hydrophobic residue inserts such as LeuGlu or Met are necessary for processing of A4CT to βA4 and should preferably be included between the signal peptide and A4CT coding regions and will remain attached to the processed A4CT.

The invention also relates to mammalian cells expressing the construct and to the DNA construct itself and vectors containing it.

Generation of transgenic mammals of the invention may be carried out conventionally, for example as described in WO93/14200, WO91/19810, WO93/02189, WO89/00689, WO92/06187, EP0451700, WO92/13069 and WO89/06689.

The APP or A4CT coding DNA is obtained by probing a human cDNA library. Mutations may be introduced by site-directed mutagenesis or during construction of the coding DNA from appropriate fragments.

Suitable promoters for use in the present invention include: Human APP (ref. 5); rat neuron specific enolase (neurons) (ref. 18); human ß actin (ref. 19); human PDGFβ (ref. 20); mouse Thy 1 (ref. 21); mouse Prion protein promoter (PrP) (ref. 14); rat synapsin 1 (brain) (ref. 22); human FMR1 (brain) (ref. 23); human neurofilament low (ref. 24), middle (brain) (ref. 25); NEX-1 (brain) (ref. 26); mouseAPLP2 (brain) (ref. 27); rat alpha tubulin (ref. 28); mouse transferrin (ref. 29); mouse HMGCR (3-hydroxy-3-methylglutaryl coenzyme A reductase, oligodendrocytes) (ref. 30) and mouse myelin basic protein (ref. 31).

A tetracycline-inducible system may also be used, which has the advantage of regulating the gene expression (induction/repression) (refs. 33, 32). This system uses two constructs: a minimal promoter (PhCMV*-1) fused to seven tetracyclic operator sequences and the cDNA in question; and a trangene containing the tetracycline-controlled tran-activator protein (tTA) coding sequence under the control of a promoter, for example taken from the above list. Each construct is used to generate a transgenic mouse. Crossing the two homozygous mice generates a double transgenic line which expresses the tTA according to the chosen promoter. This tTA induces expression of the cDNA by activating the PhCMV*-1, but only in the absence of tetracycline. In the presence of tetracycline there is only basal expression.

A preferred promoter is the mouse Prion protein promoter (ref. 14)

The construct is prepared by conventional recombinant DNA techniques (ref. 10).

The transgenic mammal is produced by conventional techniques (refs. 8, 15, 16, 17).

In one aspect, the transgenic mammal is produced by introduction of the construct into an embryo, insertion of the embryo into a surrogate mother and allowing the embryo to develop to term.

The construct is prepared for transfer to the host animal by cleavage of vector containing the construct and purification of the DNA (ref. 8)

The transfer is carried out conventionally preferably using microinjection as described in detail in reference 8.

In an alternative aspect the transgenic mammal is produced by introduction of the construct into embryonic stem cells by conventional methods such as calcium phosphate/DNA precipitation, direct injection or electroporation (ref. 9) followed by injection of the transformed cells into blastocytes and insertion of the resulting embryo into a surrogate mother as described above.

Transgenic animals are identified by DNA analyis using Southern blot and PCR to detect founder animals.

The transgenic mammal is preferably a rodent such as rat or mouse, more preferably a mouse.

Mammalian cells expressing the construct may be prepared by conventional methods.

Host cells are genetically engineered (transduced or transformed or transfected) with the vectors of this invention which may be, for example, a cloning vector or an expression vector. The vector may be, for example, in the form of a plasmid, a viral particle, a phage, etc. The engineered host cells can be cultured in conventional nutrient media modified as appropriate for activating promoters, selecting transformants or amplifying the human genes. The culture conditions, such as temperature, pH and the like will be apparent to the ordinarily skilled artisan.

Various mammalian cell culture systems can be employed to express recombinant protein. Examples of mammalian expression systems include the COS-7 lines of monkey kidney fibroblasts, described by Gluzman, Cell, 23:175 (1981), and other cell lines capable of expressing a compatible vector, for example, the SH-SY5Y, CHO and HeLa cell lines.

The selection of an appropriate host is deemed to be within the scope of those skilled in the art from the teachings herein.

Mammalian expression vectors will comprise an origin of replication, a suitable promoter and enhancer, and also any necessary ribosome binding sites, polyadenylation site, splice donor and acceptor sites, transcriptional termination sequences, and 5' flanking nontranscribed sequences. DNA sequences derived from the SV40 splice, and polyadenylation sites may be used to provide the required nontranscribed genetic elements.

In addition, the expression vectors preferably contain one or more selectable marker genes to provide a phenotypic trait for selection of transformed host cells such as hygromycin or neomycin resistance for eukaryotic cell culture.

The appropriate DNA sequence may be inserted into the vector by a variety of procedures. In general, the DNA sequence is inserted into an appropriate restriction endonuclease site(s) by procedures known in the art. Such procedures and others are deemed to be within the scope of those skilled in the art.

The DNA sequence in the expression vector is operatively linked to an appropriate expression control sequence(s) (promoter) to direct mRNA synthesis. Examples of such promoters include the CMV promoter, pCEP4 (Invitrogen) and other promoters known to control expression of genes in eukaryotic cells or their viruses and replicable and viable in the host.

Introduction of the construct into the host cell can be effected by calcium phosphate transfection, lipofectin-mediated transfection, or electroporation. (Davis, L., Dibner, M., Battey, I., Basic Methods in Molecular Biology, (1986)).

The transgenic mammal or cells of the invention may be used to screen for drugs which inhibit deposit of βA4 by administering test drug to the mammal or cell culture medium and observing changes in APP expression and processing, histopathology and/or behavioural changes. The invention extends to such method of screening.

Suitable techniques for making such observations are described in WO93/14200.

Examples

The following constructs with sequences given in Table 2 were prepared by site directed mutagenesis of wtSPA4CT (ref.1):

(1) SPA4CT T43S (SEQ ID NOs: 1 and 2)

(2) SPA4CT T43A (SEQ ID NOs: 3 and 4)

(3) SPA4CT T43F (SEQ ID NOs: 5 and 6)

(4) SPA4CT T43A+V46F (SEQ ID NOs: 7 and 8)

(5) SPA4CT T43G+V46F (SEQ ID NOs: 9 and 10)

(6) SPA4CT C-terminal insertion ('CTI')in the transmembrane domain (LV between T48 and L49) (SEQ ID NOs: 11 and 12)

as well as a construct carrying the FAD mutation SPA4CT V46F.

wtSPA4CT consists of the 17 amino acid long signal peptide of APP followed by two additional amino acids of APP 695 (Leu and Glu) and then continuing with the βA4 sequence and the whole C-terminal domain and the mutagenesis was carried out in vector pSP65/SPA4CT (ref. 11). Additionally the mutagenesis leading to construct (3) SPA4CT T43F was carried out in pBS/SPA4CT rev. pBS/SPA4CT rev was obtained by cloning the KpnI/Nhe fragment of pCEP/SPA4CT (ref. 2) in the pBS/SPC99 vector (ref. 34) that was digested with KpnI/XbaI.

The constructs were inserted into pCEP vector (ref. 2) and were stably transfected into COS7 cells.

Biological activity

Measurement of βA4 in the conditioned medium

Stably transfected COS7 cells were metabolically labeled over night in methionine free MEM-medium containing 10% FCS and 133 µCi/ml ^{35}S-methionine. βA4 and A4CT were immunoprecipated, separated on a 10% Tris-Tricine gel and quantified by phosphorimaging. The following antibodies were used:

G2-10 (monoclonal) against synthetic peptide βA4 33-40 for the immunoprecipitation of $\beta A4_{n-40}$,

G2-11 (monoclonal) against synthetic peptide βA4 35-42 for the immunoprecipitation of $\beta A4_{n-42}$,

692 (polyclonal rabbit serum) against synthetic peptide βA4 1-40 for the immunoprecipitation of βA4. n-40 and n-42 stand for peptides with a defined C-terminus (ie residue 40 or 42 respectively of βA4) but allowing for possible N-terminal homogeneity. The full length βA4 forms produced by the particular constructs described herein contain Leu, Glu at positions -2, -1.

Detection of A4CT in the cell lysate

The stably transfected COS7 cells were metabolically labeled for 10 min in methionine free MEM-medium containing 133 µCi/ml ^{35}S-methionine. In the cell lysate A4CT was immunoprecipitated with polyclonal antibody against A4CT (ref. 2), separated on a 10% Tris-Tricine gel and quantified by phosphorimaging.

Results

Expression of A4CT

All constructs (wt SPA4CT and mutated SPA4CT) were expressed in similar amounts. The signal peptide of SPA4CT was completely removed leading to Leu-Glu-A4CT.

Release of βA4

All A4CT constructs were processed to βA4 and produce similar amounts of βA4.

Generation of $\beta A4_{1-42}$ and $\beta A4_{1-40}$

For all the constructs both βA4 species $\beta A4_{1-42}$ and $\beta A4_{1-40}$ were released. The ratio $\beta A4_{1-42}/\beta A4_{1-40}$ was determined and the results are shown in Table 1.

Table 1

SPA4CT	$bA4_{1-42}/bA4_{1-40}$	increase of $bA4_{1-42}/bA4_{1-40}$ relative to wt SPA4CT by factor	significance p (student's t test)
wildtype	4.7 ± 1.3	1.0	
V46F	17.1 ± 4.8	3.6	< 0.001
(1) T43S	7.7 ± 1.2	1.6	0.01
(2) T43A	10.7 ± 2.3	2.2	<0.02
(3) T43F	9.5 ± 1.5	2.0	<0.001
(4) T43A+V46F	26.5 ± 3.6	5.6	< 0.001
(5) T43G+V46F	27.5 ± 4.7	5.8	<0.001
(6) CTI	19.6 ± 2.2	4.1	<0.001

Conclusions

The FAD linked mutation Val(717)Phe (Val(46)Phe of A4CT) is known to lead to a higher ratio of $\beta A4_{1-42}$/ $\beta A4_{1-40}$ for both SPA4CT and APP compared with the wildtype proteins.

The above results demonstrate that SPA4CT expressing cells (COS7) generate the same βA4 species ($\beta A4_{1-40}$ and $\beta A4_{1-42}$) as APP expressing cells. This suggests that the mechanism of βA4 generation is the same in APP and SPA4CT expressing cells.

The mutations near the C-terminus of βA4 are able to influence the γ-cleavage site, whereas the overall amount of generated βA4 as well as the ratio of βA4/p3 remain unchanged.

The single mutants (1), (2) and (3) have an increased ratio $\beta A4_{1-42}/\beta A4_{1-40}$ relative to wt. The double mutants (4), (5) and (6) lead, respectively, to a 5.6-, 5.8 and 4.1-fold increase in $\beta A4_{1-42}/\beta A4_{1-40}$ relative to wt.

Generation of transgenic mice expressing SPA4CT T43A+V46F

The above described constructs which produce enhanced production of $\beta A4_{1-42}$.relative to $\beta A4_{1-40}$ are useful for the generation of transgenic mice developing

amyloid plaques. By way of example, the construct SPA4CT T43A+V46F driven by the mouse Prion protein promoter (ref. 14) is used to transform a mouse by the following procedures:

The construct is prepared and purified.

Female mice are induced to superovulate and embryos are recovered.

DNA is microinjected into the pronucleus of embryos.

Embryos are transferred into pseudopregnant mice (female mice previously paired with vasectomised males).

Embryos are developed and mice are born.

Founder mice are identified by Southern blot and PCR and bred on.

Suitable mice lines are as follows:

Donor mice (embryos for pronucleus injection): B6D2F2

Acceptor mice: NMRI

Mice for further breeding: C57BL6

Screening of drugs using transgenic mice

The transgenic mice described above may be used to screen for potential activity of test drugs in the treatment of Alzheimer's disease.

APP expression and processing may be examined using detection of mRNA by Northern blots and detection of polypeptides using polyclonal and monoclonal antibodies that are specific to the terminal regions of the target peptides.

Histopathological observations may be made using immunohistological techniques to permit identification of amyloid plaques and in situ hybridisation using labelled probes to target mRNA.

Observation of behavioural changes may employ conventional tests used to assess learning and memory deficits.

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Table 2: Sequence listings

(1) T43S

Protein
SEQ ID NO: 1
MLPGLALLLLAAWTARALEDAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMVGGVVIASVIVITLVMLK
KKQYTSIHHGVVEVDAAVTPEERHLSKMQQNGYENPTYKFFEQMQN

DNA
SEQ ID NO: 2
ATGCTGCCCGGTTTGGCACTGCTCCTGCTGGCCGCCTGGACGGCTCGGGCGCTGGAGGATGCAGAATTCCGA
CATGACTCAGGATATGAAGTTCATCATCAAAAATTGGTGTTCTTTGCAGAAGATGTGGGTTCAAACAAAGGT
GCAATCATTGGACTCATGGTGGGCGGTGTTGTCATAGCTAGCGTGATCGTCATCACCTTGGTGATGCTTAAG
AAGAAACAGTACACATCCATTCATCATGGTGTGGTGGAGGTTGACGCCGCTGTCACCCCAGAGGAGCGCCAC
CTGTCCAAGATGCAGCAGAACGGCTACGAAAATCCAACCTACAAGTTCTTTGAGCAGATGCAGAAC

(2) T43A

Protein
SEQ ID NO: 3
MLPGLALLLLAAWTARALEDAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMVGGVVIAAVIVITLVMLK
KKQYTSIHHGVVEVDAAVTPEERHLSKMQQNGYENPTYKFFEQMQN

DNA
SEQ ID NO: 4
ATGCTGCCCGGTTTGGCACTGCTCCTGCTGGCCGCCTGGACGGCTCGGGCGCTGGAGGATGCAGAATTCCGA
CATGACTCAGGATATGAAGTTCATCATCAAAAATTGGTGTTCTTTGCAGAAGATGTGGGTTCAAACAAAGGT
GCAATCATTGGACTCATGGTGGGCGGTGTTGTCATAGCGGCAGTGATCGTCATCACCTTGGTGATGCTTAAG
AAGAAACAGTACACATCCATTCATCATGGTGTGGTGGAGGTTGACGCCGCTGTCACCCCAGAGGAGCGCCAC
CTGTCCAAGATGCAGCAGAACGGCTACGAAAATCCAACCTACAAGTTCTTTGAGCAGATGCAGAAC

(3) T43F

Protein
SEQ ID NO: 5
MLPGLALLLLAAWTARALEDAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMVGGVVIAFVIVITLVMLK
KKQYTSIHHGVVEVDAAVTPEERHLSKMQQNGYENPTYKFFEQMQN

DNA
SEQ ID NO: 6
ATGCTGCCCGGTTTGGCACTGCTCCTGCTGGCCGCCTGGACGGCTCGGGCGCTGGAGGATGCAGAATTCCGA
CATGACTCAGGATATGAAGTTCATCATCAAAAATTGGTGTTCTTTGCAGAAGATGTGGGTTCAAACAAAGGT
GCAATCATTGGACTCATGGTGGGCGGTGTTGTCATAGCATTCGTGATCGTCATCACCTTGGTGATGCTGAAG
AAGAAACAGTACACATCCATTCATCATGGTGTGGTGGAGGTTGACGCCGCTGTCACCCCAGAGGAGCGCCAC
CTGTCCAAGATGCAGCAGAACGGCTACGAAAATCCAACCTACAAGTTCTTTGAGCAGATGCAGAAC

(4) T43A+V46F

Protein
SEQ ID NO: 7

MLPGLALLLLAAWTARALEDAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMVGGVVIAAVIEITLVMLK
KKQYTSIHHGVVEVDAAVTPEERHLSKMQQNGYENPTYKFFEQMQN

DNA
SEQ ID NO: 8
ATGCTGCCCGGTTTGGCACTGCTCCTGCTGGCCGCCTGGACGGCTCGGGCGCTGGAGGATGCAGAATTCCGA
CATGACTCAGGATATGAAGTTCATCATCAAAAATTGGTGTTCTTTGCAGAAGATGTGGGTTCAAACAAAGGT
GCAATCATTGGACTCATGGTGGGCGGTGTTGTCATAGCGGCAGTAATATTCATCACCTTGGTGATGCTTAAG
AAGAAACAGTACACATCCATTCATCATGGTGTGGTGGAGGTTGACGCCGCTGTCACCCCAGAGGAGCGCCAC
CTGTCCAAGATGCAGCAGAACGGCTACGAAAATCCAACCTACAAGTTCTTTGAGCAGATGCAGAAC

(5) T43G+V46F

Protein
SEQ ID NO: 9
MLPGLALLLLAAWTARALEDAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMVGGVVIAGVIEITLVMLK
KKQYTSIHHGVVEVDAAVTPEERHLSKMQQNGYENPTYKFFEQMQN

DNA
SEQ ID NO: 10
ATGCTGCCCGGTTTGGCACTGCTCCTGCTGGCCGCCTGGACGGCTCGGGCGCTGGAGGATGCAGAATTCCGA
CATGACTCAGGATATGAAGTTCATCATCAAAAATTGGTGTTCTTTGCAGAAGATGTGGGTTCAAACAAAGGT
GCAATCATTGGACTCATGGTGGGCGGTGTTGTCATAGCCGGCGTAATATTCATCACCTTGGTGATGCTTAAG
AAGAAACAGTACACATCCATTCATCATGGTGTGGTGGAGGTTGACGCCGCTGTCACCCCAGAGGAGCGCCAC
CTGTCCAAGATGCAGCAGAACGGCTACGAAAATCCAACCTACAAGTTCTTTGAGCAGATGCAGAAC

(6) CTI

Protein
SEQ ID NO: 11
MLPGLALLLLAAWTARALEDAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMVGGVVIATVIVITLVLVM
LKKKQYTSIHHGVVEVDAAVTPEERHLSKMQQNGYENPTYKFFEQMQN

DNA
SEQ ID NO: 12
ATGCTGCCCGGTTTGGCACTGCTCCTGCTGGCCGCCTGGACGGCTCGGGCGCTGGAGGATGCAGAATTCCGA
CATGACTCAGGATATGAAGTTCATCATCAAAAATTGGTGTTCTTTGCAGAAGATGTGGGTTCAAACAAAGGT
GCAATCATTGGACTCATGGTGGGCGGTGTTGTCATAGCGACAGTGATCGTCATCACCCTAGTACTCGTGATG
CTGAAGAAGAAACAGTACACATCCATTCATCATGGTGTGGTGGAGGTTGACGCCGCTGTCACCCCAGAGGAG
CGCCACCTGTCCAAGATGCAGCAGAACGGCTACGAAAATCCAACCTACAAGTTCTTTGAGCAGATGCAGAAC

Claims

1. A construct comprising a human APP or A4CT DNA sequence encoding a mutation selected from:
(i) T43A, T43S, T43G, T43V, T43L, T43I or T43F; and
(ii) Insertion in the transmembrane domain (residues 43 to 52) of a sequence of 1 to 10 amino acid residues;
(numbering relative to A4CT) operably linked to a promoter sequence.

2. A construct according to claim 1 wherein the construct encodes mutations (i) and (ii).

3. A construct according to claim 1 or 2 wherein mutation (i) is selected from T43A and T43S.

4. A construct according to any preceding claim wherein insertion (ii) is located between T48 and L49.

5. A construct according to any preceding claim wherein the residues for insertion (ii) are selected from F, I, G, Y, L, A, P, W, M, S, T, N and Q.

6. A construct according to any preceding claim wherein the insertion (ii) is 2 to 6 residues long.

7. A construct according to claim 6 wherein the insertion (ii) is LV.

8. A construct according to any preceding claim wherein the construct additionally encodes a mutation selected from:
V46F, V46I, V46G, V46Y, V46L, V46A, V46P, V46W, V46M, V46S, V46T, V46N or V46Q.

9. A construct according to any preceding claim wherein the construct further encodes the APP signal sequence (APP residues 1 to 17) immediately upstream of the APP or A4CT DNA sequence together with hydrophobic residue inserts between the signal peptide and A4CT coding regions.

10. A construct according to claim 1 comprising a sequence selected from:
(1) SPA4CT T43S (SEQ ID NO: 2)
(2) SPA4CT T43A (SEQ ID NO: 4)
(3) SPA4CT T43F (SEQ ID NO: 6)
(4) SPA4CT T43A+V46F (SEQ ID NO: 8)
(5) SPA4CT T43G+V46F (SEQ ID NO: 10)
(6) SPA4CT C-terminal insertion ('CTI')in the transmembrane domain (LV between T48 and L49) (SEQ ID NO: 12)

11. A construct according to any preceding claim comprising a mammalian promoter selected from Human APP: rat neuron specific enolase (neurons); human ß actin; human

PDGFβ; mouse Thy 1; mouse Prion protein promoter (PrP); rat synapsin 1 (brain); human FMR1 (brain); human neurofilament low, middle (brain); NEX-1 (brain); mouseAPLP2 (brain); rat alpha tubulin; mouse transferrin; mouse HMGCR (3-hydroxy-3-methylglutaryl coenzyme A reductase, oligodendrocytes) and mouse myelin basic protein.

12. A non-human transgenic mammal whose cells contain a construct according to any preceding claim.

13. A transgenic mammal according to claim 12 which is a rodent.

14. A mammalian host cell expressing the construct of any of claims 1 to11.

15. A vector containing the construct of any of claims 1 to 11.

16. A method of screening for drugs which inhibit deposit of βA4 by administering test drug to the transgenic mammal of claim 12 or 13 or cell culture medium containing the mammalian host cell of claim 14 and observing changes in APP expression and processing, histopathology and/or behavioural changes.

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(57) Abstract

Constructs comprising a human APP or A4CT DNA sequence encoding mutations which lead to a higher ratio of $\beta A4_{1-42}/\beta A4_{1-40}$ than wild type and their use in the production of transgenic animals developing amyloid plaques as a model of Alzheimer's disease.

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INTERNATIONAL SEARCH REPORT

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A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/00 A01K67/027 C07K14/47 C12N15/12 C12N5/10

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B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A01K C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	LICHTENTHALER, S. ET AL.: "Mutations near the C-terminus of beta-A4 influence the gamma cleavage of A4CT (C99) in COS7 cells" NEUROBIOLOGY OF AGING, vol. 17, no. 4 sup, 19 July 1996, pages s130-s131, XP002056699	1,3,9,10
Y	see abstract 524	8,9, 12-16

Y	WO 93 14200 A (TSI CORP) 22 July 1993 cited in the application see the whole document	8,9, 12-16

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Date of the actual completion of the international search	Date of mailing of the international search report
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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
T	LICHTENTHALER, S.F. ET AL.: "Mutations in the transmembrane domain of APP altering gamma-secretase specifity" BIOCHEMISTRY., vol. 36, no. 49, 9 December 1997, EASTON, PA US, pages 15396-15403, XP002056700 see the whole document	1-10

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INTERNATIONAL SEARCH REPORT

Information on patent family members

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PCT/EP 97/03960

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